

[0139] The following steps (FIG. 13) were performed in constructing the pDElia2<sub>FC5</sub>-KDB2 vector:

- [0140] 1. pGEMT-ask-asd: an approximately 2.6 Kb PCR product containing the ask-asd operon of ATCC21529 using primers ask and asd was cloned into pGEM-T (Promega pGEM-T vector systems).
- [0141] 2. pUC18-ddh: an approximately 1.3 Kb KpnI fragment of pADM21 containing ddh (BF100 locus) was subcloned into pUC18 at the KpnI site.
- [0142] 3. pFC3-ask-asd: an approximately 2.6 Kb NsiI-ApaI fragment of pGEMT-ask-asd was cloned into pFC3 cut with PstI and ApaI.
- [0143] 4. pFC1-dapB-ORF2: an approximately 2 Kb PCR product of NRRL-B11474 dapB-ORF2 coding region was cloned into pFC1 at the EcoRV site.
- [0144] 5. pFC1-ddh: an approximately 1.3 Kb PstI-EcoRI fragment of pUC18-ddh was cloned into pFC1 cut with PstI and EcoRI.
- [0145] 6. pUC19-P1: an approximately 550 bp HpaI-PvuII fragment (containing the first promoter, P1, of the argS-lysA operon) of pRS6 was cloned into pUC19 at the SmaI site.
- [0146] 7. pUC19-P1lysA: an approximately 1.45 Kb promoterless PCR product, using primers LysA(ATG) and LysA3B, of ASO19 lysA coding region is cloned into pUC19-P1 at the HincII site.
- [0147] 8. pFC1-P1lysA: an approximately 2 Kb EcoRI-HindIII fragment of pUC9-P1lysA was cloned in to pFC1 cut with EcoRI and HindIII.
- [0148] 9. pFC1-ddh-P1lysA: an approximately 1.3 Kb EcoRI-NotI fragment of pFC1-ddh was cloned into pFC1-P1lysA cut with EcoRI and NotI.
- [0149] 10. pFC1-ask-asd-ddh-P1lysA: an approximately 2.6 Kb SwaI-FseI fragment of pFC3-ask-asd was cloned into pFC1-ddh-P1lysA cut with SwaI and FseI.
- [0150] 10. pFC1-ask-asd-dapB-ORF2-ddh-P1lysA (pFC1-KDB2HP1L): an approximately 5.9 Kb SpeI fragment of pFC1-ask-asd-ddh-P1lysA was cloned into pFC1-dapB-ORF2 at the SpeI site.
- [0151] 11. pDElia2<sub>FC5</sub>: the small PvuII fragment of pFC5 was ligated with the large PvuII fragment of pDElia2.
- [0152] 12. pDElia2<sub>FC5</sub>-ask-asd-dapB-ORF2 (pDElia2<sub>FC5</sub>-KDB2): an approximately 4.7 Kb ApaI fragment containing KDB2 of pFC1-KDB2HP1L was cloned into pDElia2<sub>FC5</sub> at the ApaI site.

[0153] Corynebacterium (NRRL-B11474) containing the pDElia2<sub>FC5</sub>-KDB2 construct was deposited at an acceptable International Depositary Authority in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The deposit has been made with the Agricultural Research Service, Culture Collection (NRRL), 1815 North University Street, Peoria, Ill. 61604 on Feb. 1, 2001. The deposit is numbered NRRL-B30459.

[0154] The following steps (FIG. 14) were performed in constructing the pDElia2<sub>FC5</sub>-KDB2HP1L vector:

- [0155] 1. pGEMT-ask-asd: an approximately 2.6 Kb PCR product containing the ask-asd operon of ATCC21529 using primers ask and asd was cloned into pGEM-T (Promega pGEM-T vector systems).
- [0156] 2. pUC18-ddh: an approximately 1.3 Kb KpnI fragment of pADM21 containing ddh (NRRL-B11474 locus) was subcloned into pUC18 at the KpnI site.
- [0157] 3. pFC3-ask-asd: an approximately 2.6 Kb NsiI-ApaI fragment of pGEMT-ask-asd was cloned into pFC3 cut with PstI and ApaI.
- [0158] 4. pFC1-dapB-ORF2: an approximately 2 Kb PCR product of NRRL-B 11474 dapB-ORF2 coding region was cloned into pFC1 at the EcoRV site.
- [0159] 5. pFC1-ddh: an approximately 1.3 Kb PstI-EcoRI fragment of pUC19-ddh was cloned into pFC1 cut with PstI and EcoRI.
- [0160] 6. pUC19-P1: an approximately 550 bp HpaI-PvuII fragment (containing the first promoter, P1, of the argS-lysA operon) of pRS6 was cloned into pUC19 at the SmaI site.
- [0161] 7. pUC19-P1lysA: an approximately 1.45 Kb promoterless PCR product, using primers LysA(ATG) and LysA3B, of ASO19 lysA coding region is cloned into pUC19-P1 at the HincII site.
- [0162] 8. pFC1-P1lysA: an approximately 2 Kb EcoRI-HindIII fragment of pUC19-P1lysA was cloned into pFC1 cut with EcoRI and HindIII.
- [0163] 9. pFC1-ddh-P1lysA: an approximately 1.3 Kb EcoRI-NotI fragment of pFC1-ddh was cloned into pFC1-P1lysA cut with EcoRI and NotI.
- [0164] 10. pFC1-ask-asd-ddh-P1lysA: an approximately 2.6 Kb SwaI-FseI fragment of pFC3-ask-asd was cloned into pFC1-ddh-P1lysA cut with SwaI and FseI.
- [0165] 11. pFC1-ask-asd-dapB-ORF2-ddh-P1lysA (pFC1-KDB2HP1L): an approximately 5.9 Kb SpeI fragment of pFC1-ask-asd-ddh-P1lysA was cloned into pFC1-dapB-ORF2 at the SpeI site.
- [0166] 12. pDElia2<sub>FC5</sub>: the small PvuII fragment of pFC5 was ligated with the large PvuII fragment of pDElia2
- [0167] 13. pDElia2<sub>FC5</sub>-ask-asd-dapB-ORF2-ddh-P1lysA (pDElia2<sub>FC5</sub>-KDB2HP1L): an approximately 7.9 Kb NHE fragment of pFC1-ask-asd-dapB-ORF2-ddh-P1lysA was cloned into pDElia2<sub>FC5</sub> at the NHE site.



[0168] Corynebacterium (NRRL-B11474) containing the pDElia2<sub>FC5</sub>-KDB2HP1L construct was deposited at an acceptable International Depositary Authority in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The deposit has been made with the Agricultural Research Service, Culture Collection (NRRL), 1815 North

11. pFC1-ask-asd-dapB-ORF2-ddh-P1lysA (pFC1-KDB2HP1L): an approximately 5.9 Kb SpeI fragment of pFC1-ask-asd-ddh-P1lysA was cloned into pFC1-dapB-ORF2 at the SpeI site.

12. pDElia2<sub>FC5</sub>: the small PvuII fragment of pFC5 was ligated with the large PvuII fragment of pDElia2

13. pDElia2<sub>FC5</sub>-ask-asd-dapB-ORF2-ddh-P1lysA (pDElia2<sub>FC5</sub>-KDB2HP1L): an approximately 7.9 Kb NHE fragment of pFC1-ask-asd-dapB-ORF2-ddh-P1lysA was cloned into pDElia2<sub>FC5</sub> at the NHE site.

[0113] Corynebacterium (NRRL-B11474) containing the pDElia2<sub>FC5</sub>-KDB2HP1L construct was deposited at an acceptable International Depository Authority in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The deposit has been made with the Agricultural Research Service, Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 on February 1, 2001. The deposit is numbered NRRL-B30522.

## EXAMPLE 2

### Screening and Selection of Strains with Improved L-Lysine Production

[0114] The production of L-lysine by cells stably transformed with multi-gene constructs is summarized in Table 1.

**Table 1. Lysine production by various parental and stably transfected bacteria**

Strain Tested	lysine titer (g/L)	L-lysine yield (%)	Cell Deposit
NRRL-B11474	31	30	
NRRL-B11474::pDElia2 <sub>FC5</sub> -KDB	34	37	NRRL-B30458